

Primer Extension Coupled with Electrospray Product Ion Mass Spectrometry

A
 PCR product: 3' TTGATCAAGTATAGGGCCGATAA^GTTCGGCATATGCGG...
 SNP primer 5' TAGTTCATATCCCGGCTATT

Step 1

50 nM PCR product, 3-4 μ M SNP primer
 1 μ M ddATP, 1 μ M ddCTP, 1 μ M ddGTP, 1 μ M ddTTP
 20 mM NH_4Ac buffer pH 8.7
 2 mM $\text{Mg}(\text{Ac})_2$
 1 unit DNA polymerase
 15-20 thermal cycles

PCR product: 3' TTGATCAAGTATAGGGCCGATAA^GTTCGGCATATGCGG...
 SNP primer 5' TAGTTCATATCCCGGCTATT^{Cdd}

Step 2

Reconstituted in 20 mM NH_4Ac buffer and
 pass sample through a metal chelating resin to
 remove magnesium from the solution.

Step 3 Analyze the sample solution using Selected Reaction Monitoring
 Electrospray Mass Spectrometry to detect the unreacted ddNTPs remaining
 in solution relative to a control sample that did not undergo primer extension.

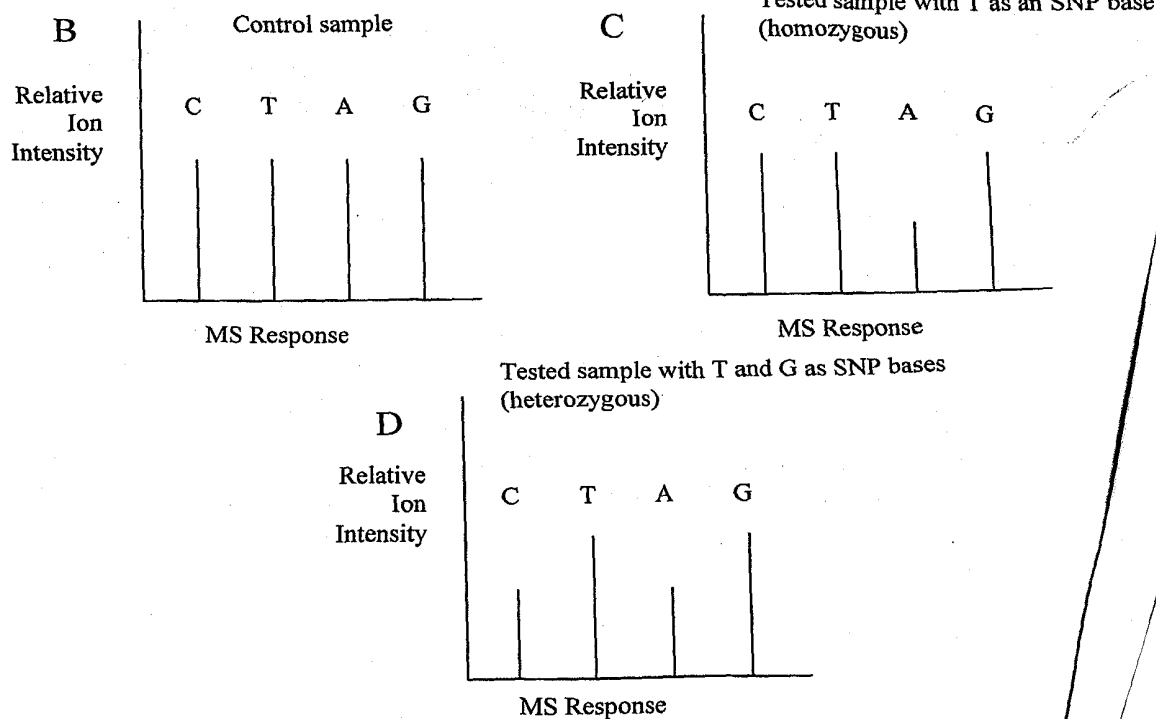


Figure 1

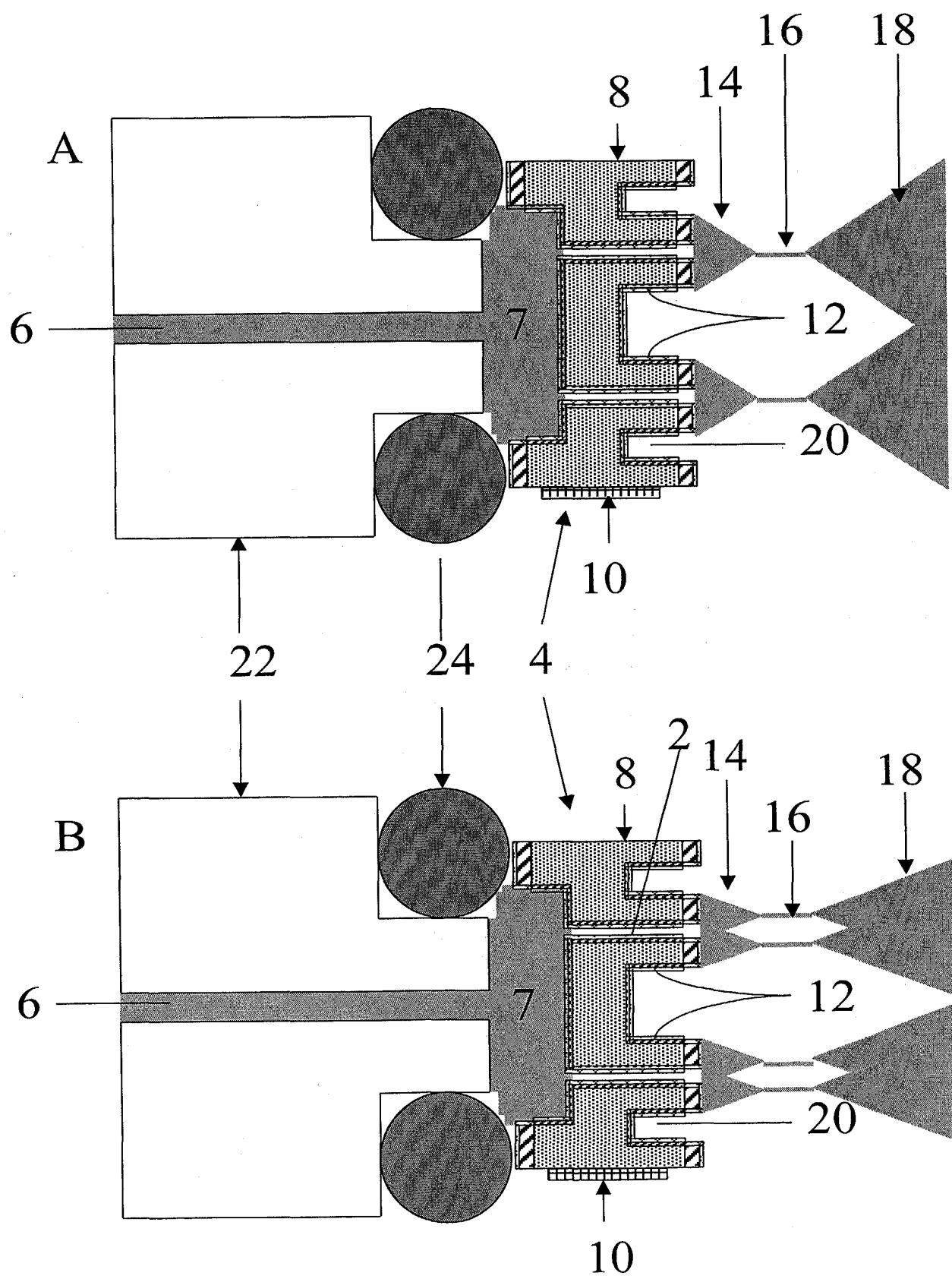


Figure 2

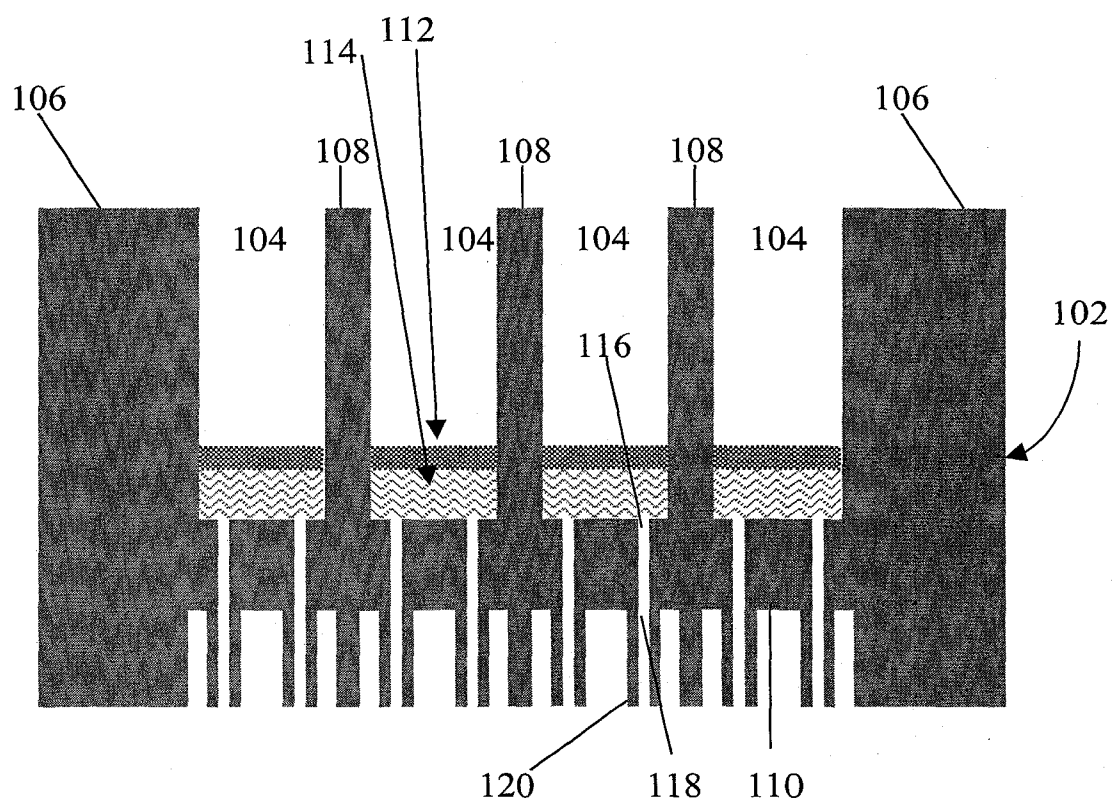


Figure 3A

[illegible]

Figure 3B

[illegible]

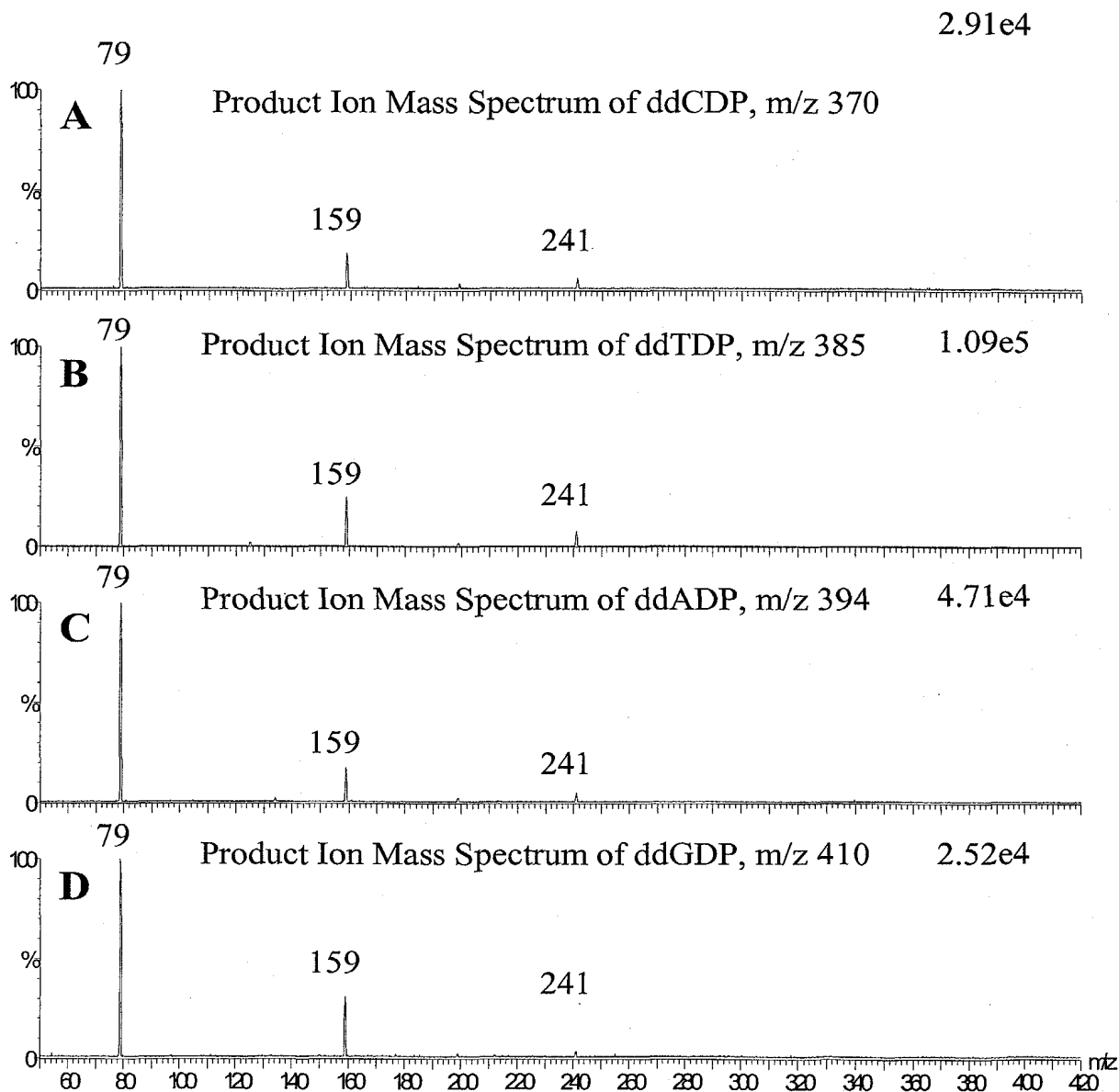


Figure 5

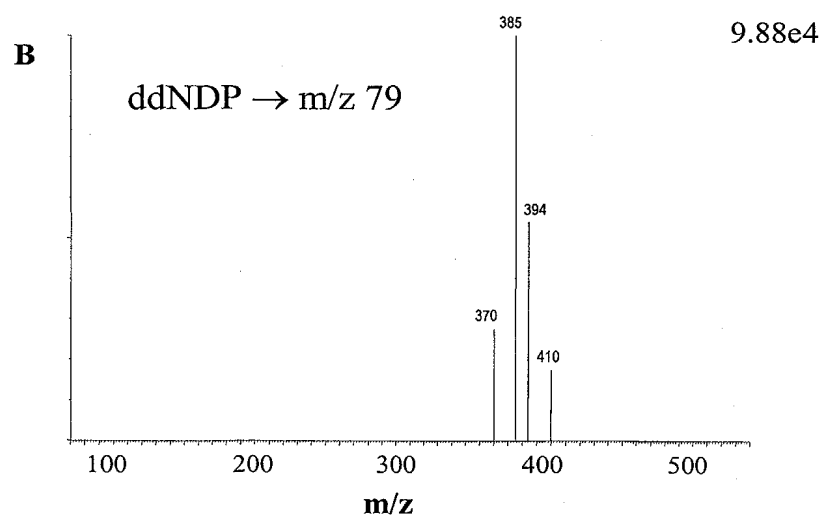
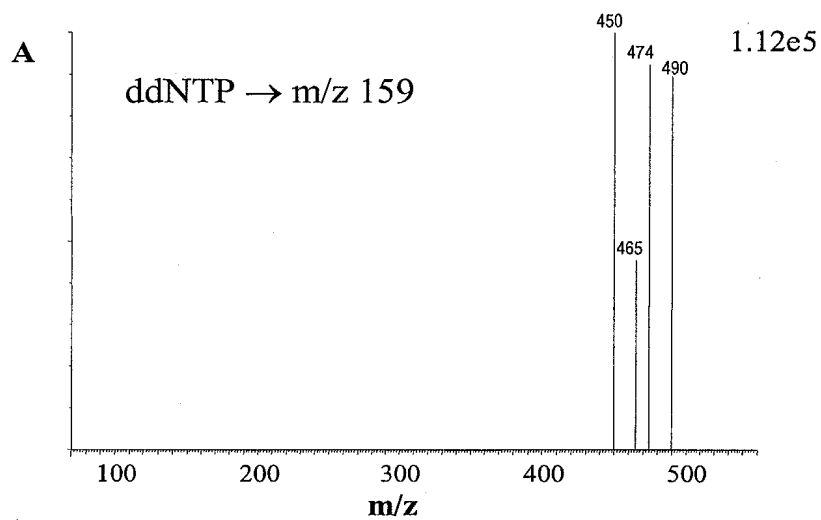


Figure 6

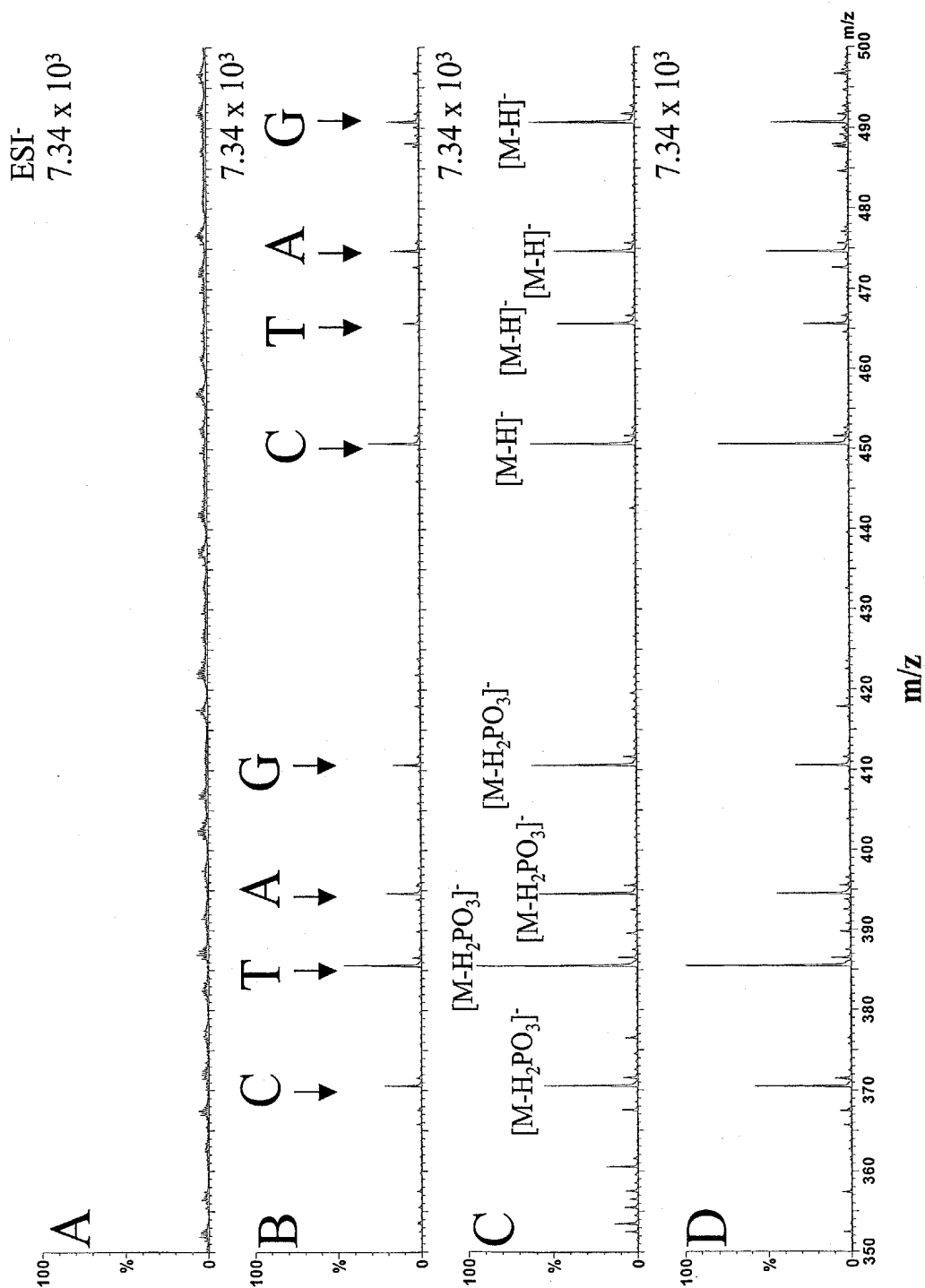


Figure 7

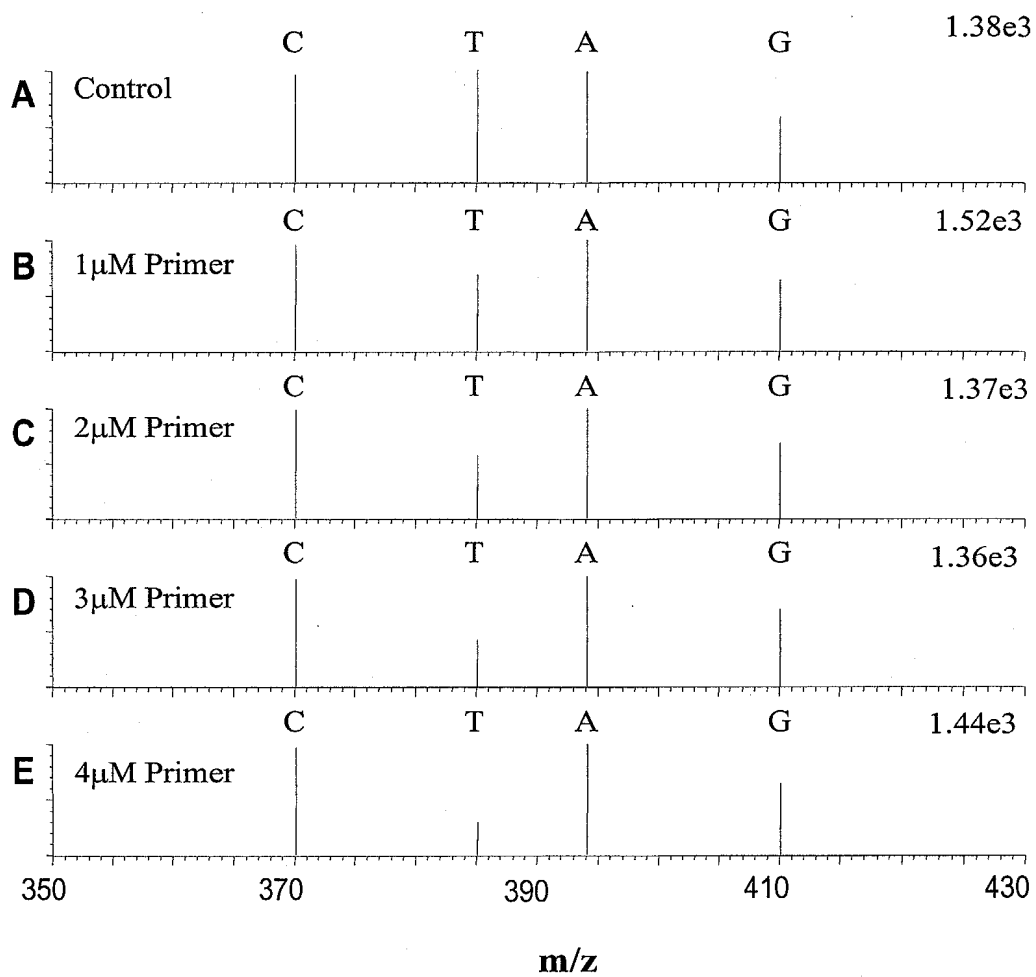


Figure 8

Schematic diagram of SNP genotyping by ESI/MS using synthetic single strand DNA as target templates in homogeneous reaction

Homogeneous templates:

Template A : 5' CCCCTGT**A**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 1)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddTTP (385.1>79)

Template C : 5' CCCCTGT**C**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 2)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddGTP (410.1>79)

Template G : 5' CCCCTGT**G**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 3)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddCTP (370.1>79)

Template T : 5' CCCCTGT**T**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 4)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddATP (394.1>79)

Figure 9

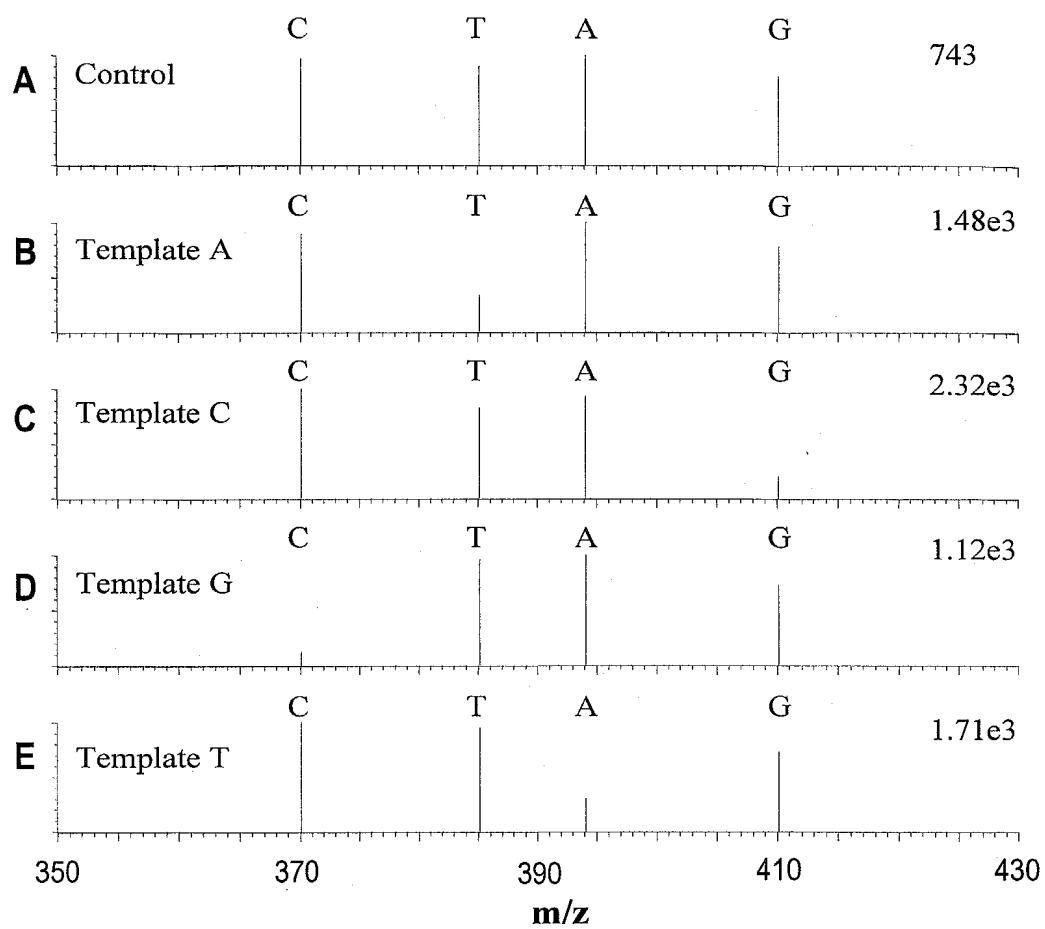


Figure 10

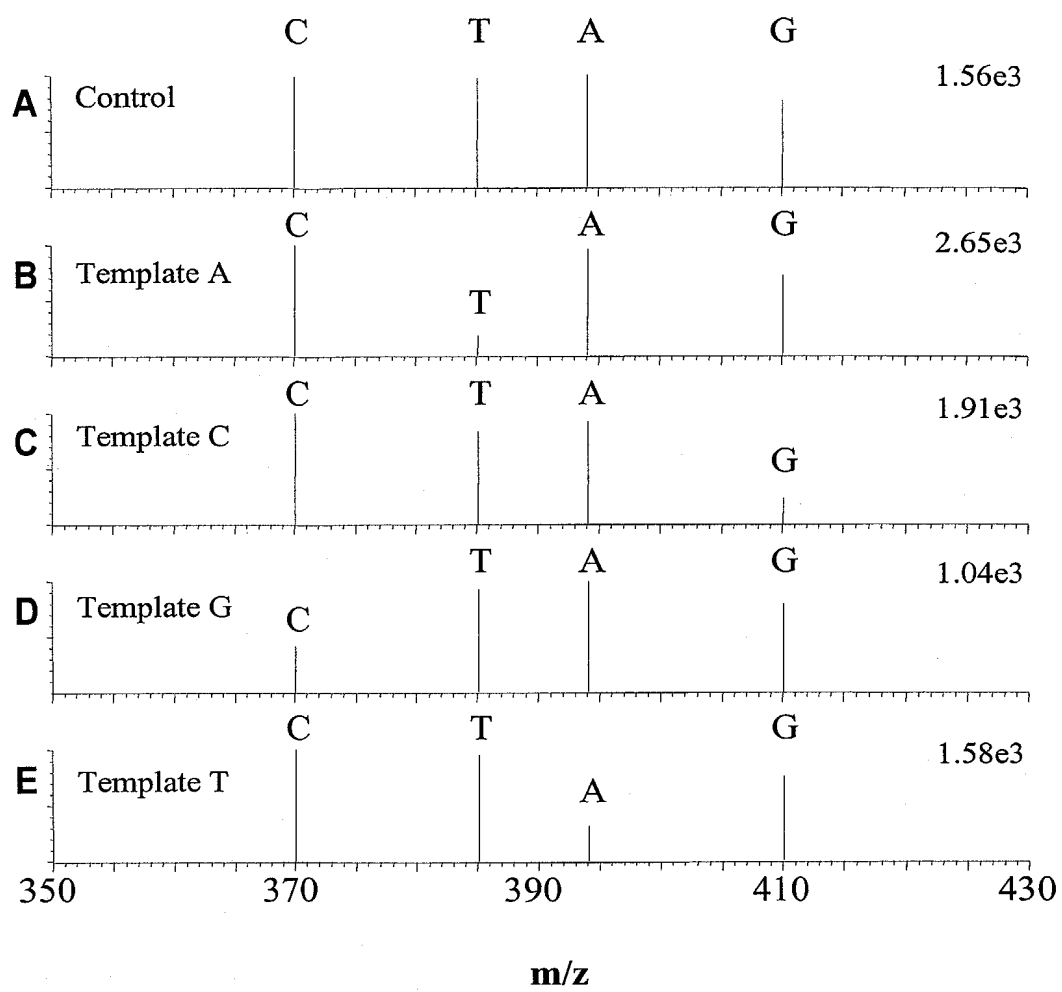


Figure 11

Schematic diagram of SNP genotyping by ESI/MS using synthetic single strand DNA as target templates in heterogeneous reaction

Heterogeneous templates (two templates mixture):

C (SEQ. ID. No. 2)

Template A+C: 5' CCCCTGT**A**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 1)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddTTP (385.1>79) and ddGTP (410.1>79)

G (SEQ. ID. No. 3)

Template A+G: 5' CCCCTGT**A**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 1)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddTTP (385.1>79) and ddCTP (370.1>79)

T (SEQ. ID. No. 4)

Template A+T: 5' CCCCTGT**A**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 1)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddTTP (385.1>79) and ddATP (394.1>79)

G (SEQ. ID. No. 3)

Template C+G: 5' CCCCTGT**C**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 2)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddGTP (410.1>79) and ddCTP (370.1>79)

T (SEQ. ID. No. 4)

Template C+T: 5' CCCCTGT**C**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 2)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddGTP (410.1>79) and ddATP (394.1>79)

T (SEQ. ID. No. 4)

Template G+T: 5' CCCCTGT**G**TCCTGTGTGAAATTGTTATCCGCTC 3' (SEQ. ID. No. 3)

SNP primer: 3' AGGACACACTTTAACAATAGGCGA 5' (SEQ. ID. No. 5)

Decreased ddNTPs: ddCTP (370.1>79) and ddATP (394.1>79)

Figure 12

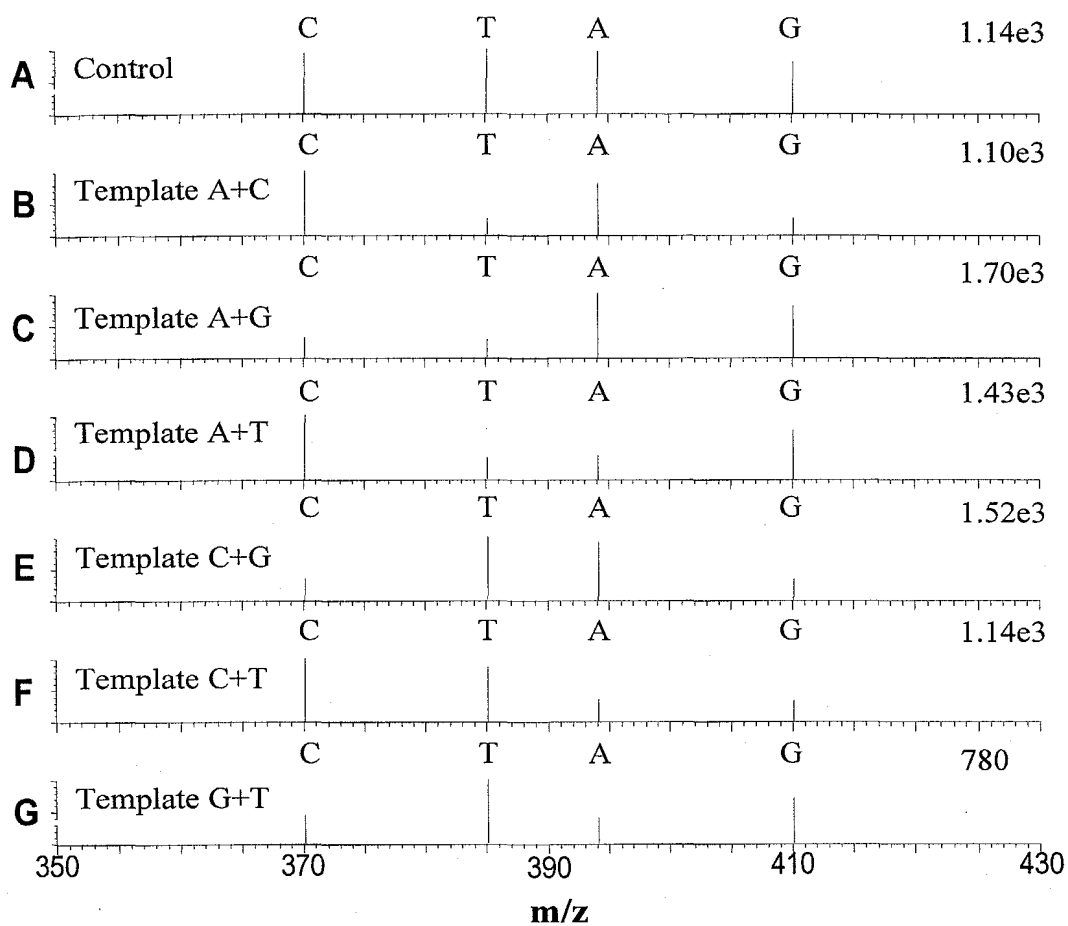


Figure 13

384 bp of partial PheA gene and its primers used for SNP assay:

384bp double-stranded target sequence: (SEQ. ID. No. 6)

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W338Ipd →
5' CGGTAATCCA TGGGAAGAGA TGTCTATCT GGATATTCAG GCCAATCTTG AATCAGCGGA
3' GCCATTAGGT ACCCTTCTCT ACAAGATAGA CCTATAAGTC CGGTTAGAAC TTAGTCGCCT

                                     T366pd → C374Apd →
AATGCAAAAA GCATTGAAAG AGTTAGGGGA AATCACCCGT TCAATGAAGG TATTGGGCTG
TTACGTTTTT CGTAACTTTC TCAATCCCCT TTAGTGGGCA AGTTACTTCC ATAACCCGAC
                                     ← C474Spu

C374Apd →
TTACCCAAGT GAGAACGTAG TGCCTGTTGA TCCAACCTGA TGAAAAGGTG CCGGATGATG
AATGGGTTCA CTCTTGCATC ACGGACAAC T AGGTTGGACT ACTTTTCCAC GGCCTACTAC
← C474Spu ← V383pu

TGAATCATCC GGCAC TGGAT TATTACTGGC GATTGTCATT CGCCTGACGC AATAACACGC
ACTTAGTAGG CCGTGACCTA ATAATGACCG CTAACAGTAA GCGGACTGCG TTATTGTGCG

GGCTTTCAC TCTGAAAACGC TGTGCGTAAT CGCCGAACCA GAATTCGAGC TCGGTACCCG
CCGAAAGTGA GACTTTTGCG ACACGCATTA GCGGCTTGGT CTTAAGCTCG AGCCATGGGC

GGGATCCTCT AGAGTCGACC TGCAGGCATG CAAGCTTGGC ACTGGCCGTC GTTTTACAAC
CCCTAGGAGA TCTCAGCTGG ACGTCCGTAC GTTCGAACCG TGACCGGCAG CAAAATGTTG

GTCGTGACTG GGAAAACCCT GGCG 3'
CAGCACTGAC CCTTTTGGGA CCGC 5'
                                     ← #1224

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Amplification primers:

W338Ipd 5'-CGGTAATCCAATTGAAGAGATGTTCT-3' (SEQ. ID. No. 7)

#1224 5'-CGCCAGGGTTTTCCAGTCACGA-3' (SEQ. ID. No. 8)

Polymorphism detection primers:

Polymorphic base

W338Ipd 5'-CGGTAATCCAATTGAAGAGATGTTCT-3'	T	(SEQ. ID. No. 7)
C374Spu 5'-TCACTTGGGTAGGATCCCAATACCTTCATT-3'	C	(SEQ. ID. No. 9)
#1224 5'-CGCCAGGGTTTTCCAGTCACGA-3'	G	(SEQ. ID. No. 8)
C374Apd 5'-AGGTATTGGGCGCCTACCCAAGTGAG-3'	T	(SEQ. ID. No. 10)
T366pd 5'-ACCCGTTCAATGAAGGTATTGGGC-3'	A	(SEQ. ID. No. 11)
V383pu 5'-AACAGGCACTACGTTCTCACTTGGGTA-3'	T	(SEQ. ID. No. 12)

Figure 14

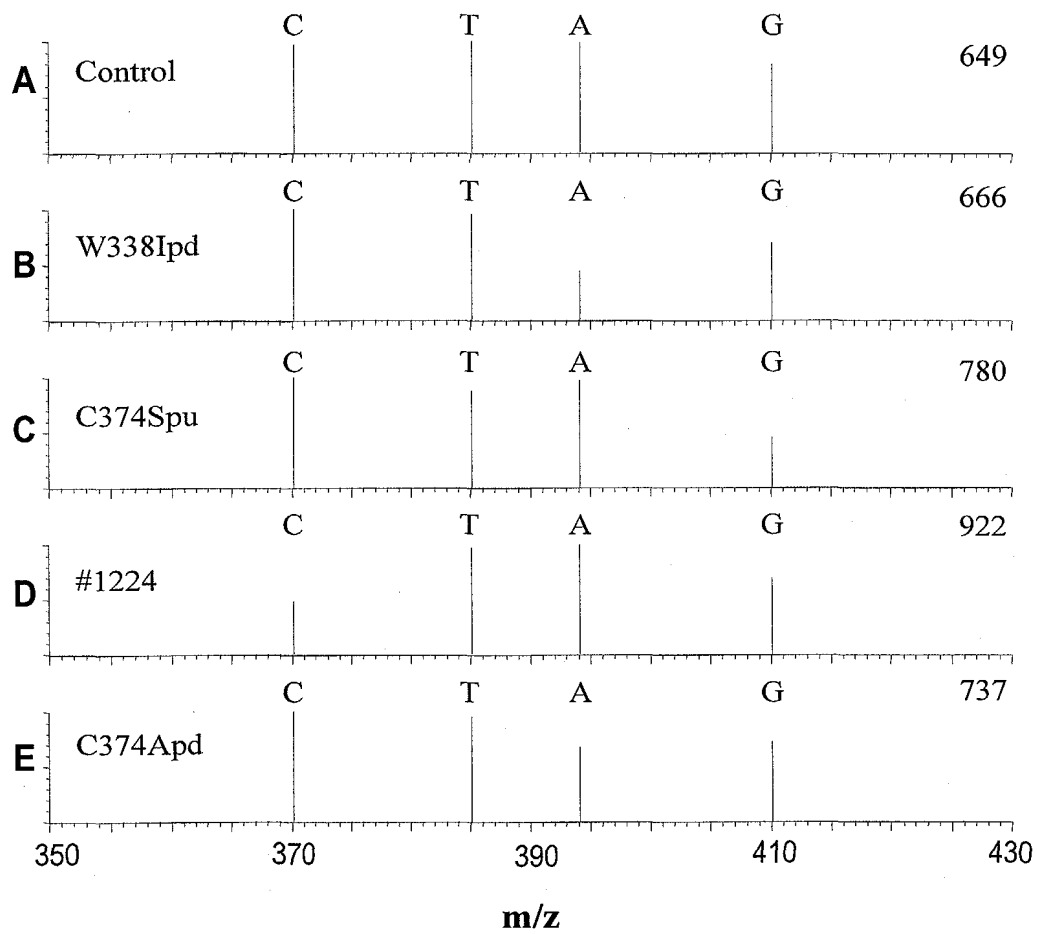


Figure 15

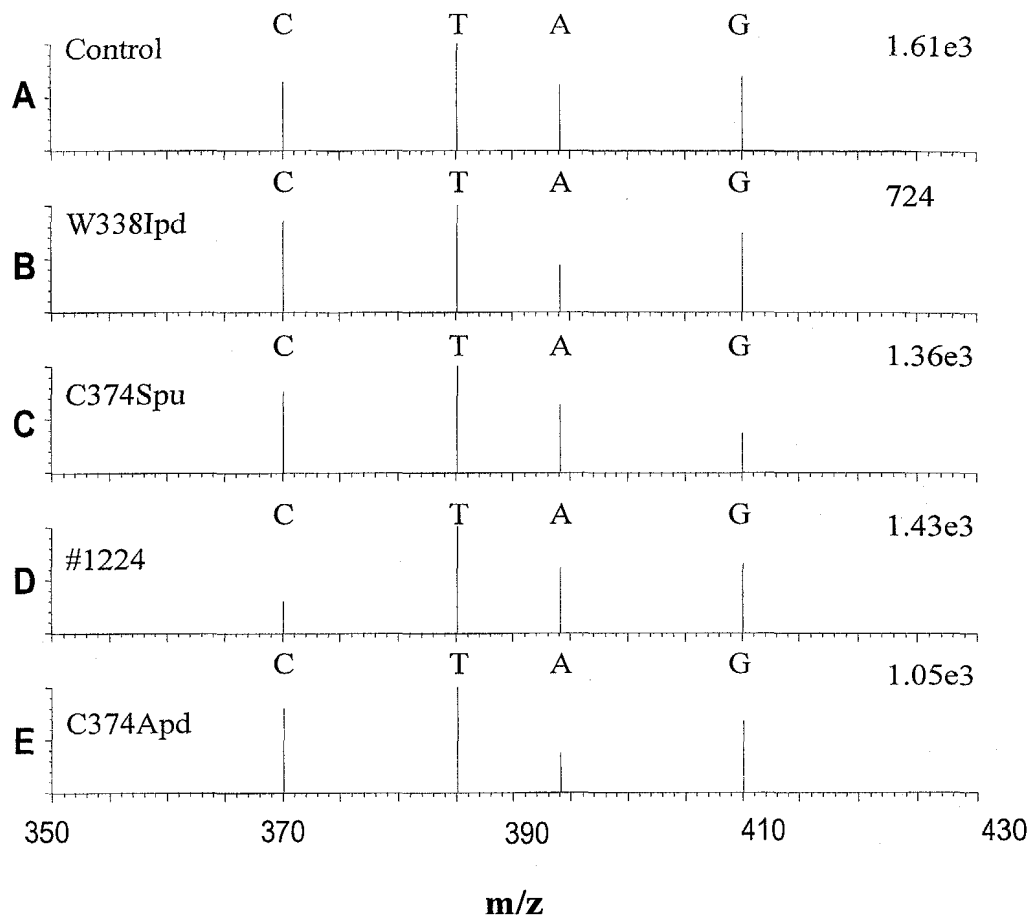


Figure 16

384 bp of partial *PheA*-C374A mutant gene and its primers used for SNP assay:

384bp double-stranded target sequence: (SEQ. ID. No. 13)

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W338Ipd →
5' CGGTAATCCA TGGGAAGAGA TGTTCTATCT GGATATTCAG GCCAATCTTG AATCAGCGGA
3' GCCATTAGGT ACCCTTCTCT ACAAGATAGA CCTATAAGTC CGGTTAGAAC TTAGTCGCCT

                                     T366pd →
AATGCAAAAA GCATTGAAAG AGTTAGGGGA AATCACCCGT TCAATGAAGG TATTGGGCGC
TTACGTTTTT CGTAACTTTC TCAATCCCCT TTAGTGGGCA AGTTACTTCC ATAACCCGAC

CTACCCAAGT GAGAACGTAG TGCCTGTTGA TCCAACCTGA TGAAAAGGTG CCGGATGATG
GATGGGTTCA CTCTTGCATC ACGGACAAC AGGTTGGACT ACTTTTCCAC GGCCTACTAC
                                     ← V383pu

TGAATCATCC GGCAC TGGAT TATTACTGGC GATTGTCATT CGCCTGACGC AATAACACGC
ACTTAGTAGG CCGTGACCTA ATAATGACCG CTAACAGTAA GCGGACTGCG TTATTGTGCG

GGCTTTCAC T GAAAAACGC TGTGCGTAAT CGCCGAACCA GAATTCGAGC TCGGTACCCG
CCGAAAGTGA GACTTTTGCG ACACGCATTA GCGGCTTGGT CTTAAGCTCG AGCCATGGGC

GGGATCCTCT AGAGTCGACC TGCAGGCATG CAAGCTTGGC ACTGGCCGTC GTTTTACAAC
CCCTAGGAGA TCTCAGCTGG ACGTCCGTAC GTTCCAACCG TGACCGGCAG CAAAATGTTG

GTCGTGACTG GGAAAACCCT GGCG 3'
CAGCACTGAC CCTTTTGGGA CCGC 5'
                                     ← #1224

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Amplification primers:

W338Ipd	5'-CGGTAATCCAATTGAAGAGATGTTCT-3'	(SEQ. ID. No. 7)
#1224	5'-CGCCAGGGTTTTCCCAGTCACGA-3'	(SEQ. ID. No. 8)

Polymorphism detection primers:

Polymorphic base

W338Ipd	5'-CGGTAATCCAATTGAAGAGATGTTCT-3'	A	(SEQ. ID. No. 7)
#1224	5'-CGCCAGGGTTTTCCCAGTCACGA-3'	C	(SEQ. ID. No. 8)
T366pd	5'-ACCCGTTCAATGAAGGTATTGGGC-3'	C	(SEQ. ID. No. 11)
V383pu	5'-AACAGGCACTACGTTCTCACTTGGGTA-3'	C	(SEQ. ID. No. 12)

Figure 17

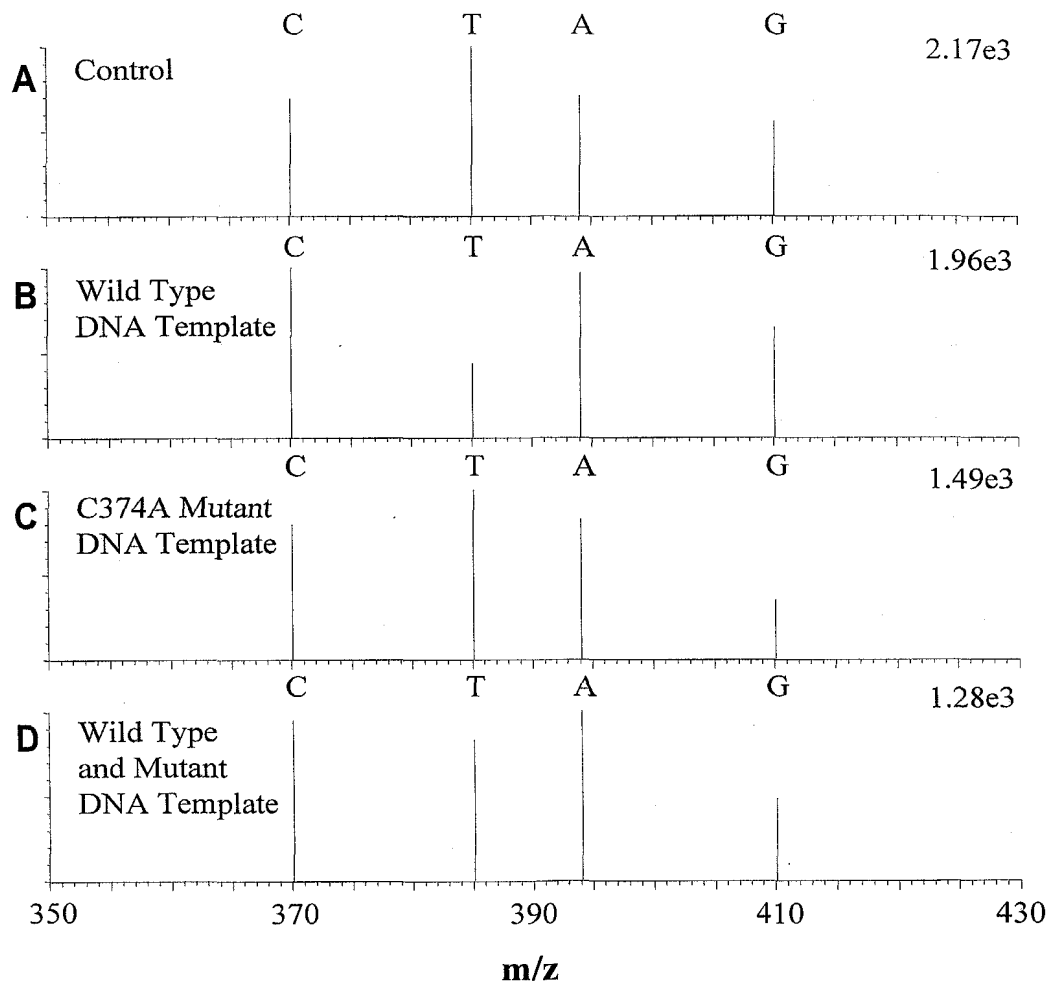


Figure 18

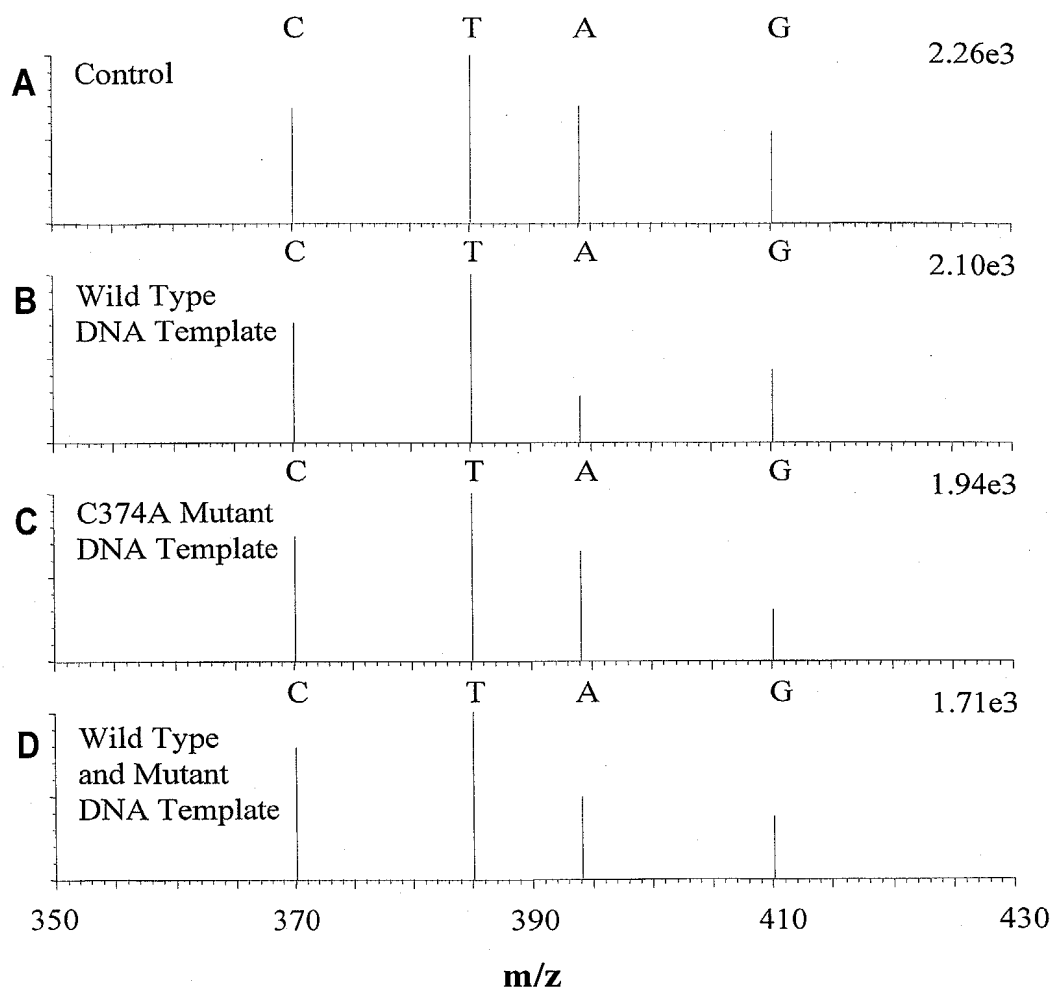


Figure 19

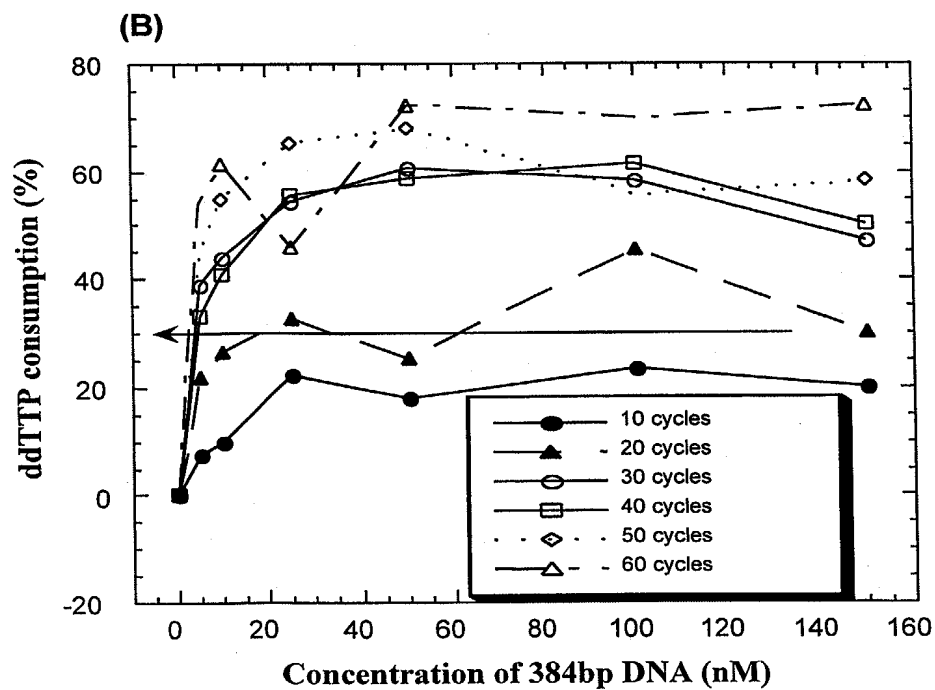
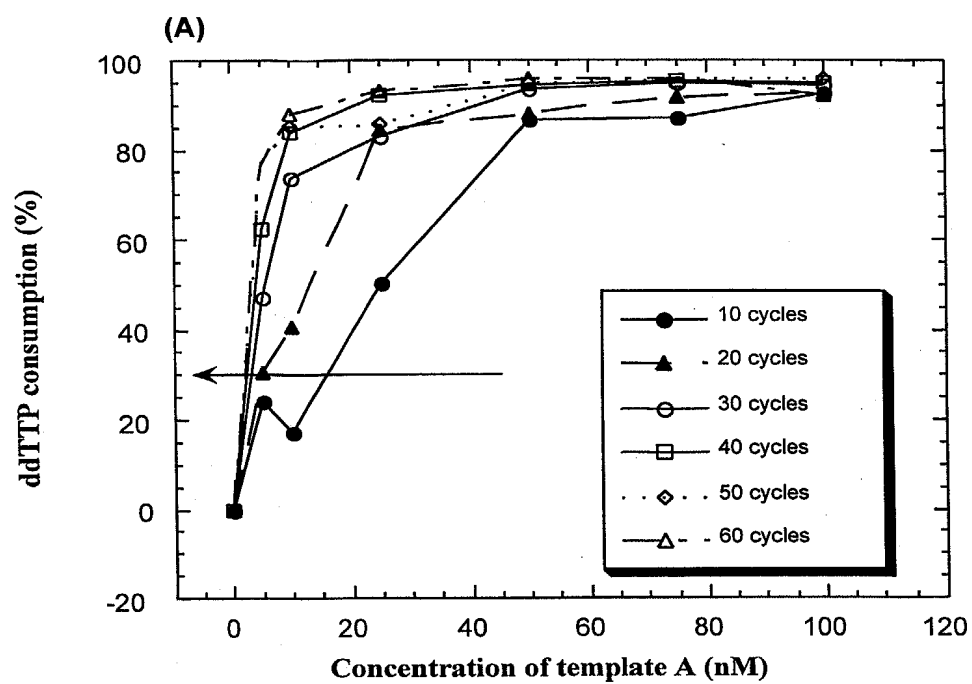


Figure 20